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RESISTANCE OF THE INFLUENZA VIRUS TO LOW TEMPERATURE AND ITS COAGULABILITY ON LIQUID AND PAPER SURFACES AND IN FOAM

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Up to the present time, a convincing series of proofs confirmed the positive role of the virus -- pathogenic for skunks, white, house, and field mice, white and wild rats (pasyukov), cats, hedgehogs, shoats, and other domestic animals -- as a primary infective agent of epidemic influenza. The lungs of sick skunks, mice, and rats were ground and emulsified in physiologic salt solution. This preparation was then filtered through gradcool membranes or waxy material, which retains the visible microbic filtrate that is active and infective against receptive animals when injected into the respiratory system.

The influenza virus can be kept in a passive state in skunks for an unlimited time; if skunks are not available, cheap test animals such as wild rats and mice can be used as hosts, and virulence increases gradually during passage.

Thus, the stock virus isolated by Smorodintsev and Drobyshevka in 1936 (A. A. Smorodintsev, Third All-Russian Conference of Microbiologists, Epidemiologists, and Specialists on Infectious Diseases, 1940) caused the death of white mice when an initial dose of 1/20 cubic centimeter of a concentration of 1:100,000 was intranasally introduced under ether anesthesia. After 150 passages (three mice in each passage) this strain appeared active in a cultivation of 1:5-10 MLD. The virus survived for 2-3 months in 50 percent glycerine at a low temperature. Turner reported that the influenza virus retains its virulence for at least 6 months in a mixture of dry ice and alcohol at a temperature of -78 degrees centigrade.

Experimental investigations on the resistance of the influenza virus to subzero temperature resulted in the production of a pure and virulent influenza virus in a high concentration which led us to the following conclusion:

We had access to liquid oxygen in unlimited quantities, and we twice conducted a series of experiments (in 1939-41 and 1946-47) in which we infected house and field mice and wild rats with the influenza virus.

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We used liquid oxygen (-183 degrees) in a Dewar flask for freezing and preserving the lung tissues of infected mice in vitro for periods of 24 hours to 7 days. We found that the lung tissues crumbled easily into a fine ice powder under the pressure of a pestle and disintegrated well into a mass of uniform consistency without the addition of quartz sand.

A suspension of this powder in physiologic salt solution and broth in a 5:1 proportion was prepared and a dose of 1/30 cubic centimeter of the centrifugates and filtrates of the suspension was administered into the respiratory system of animals (18 house rats, 12 field rats, and 9 wild rats) susceptible to the virus. The animals became infected and/or died within similar periods of time with pathological degenerations in the lungs.

Introduction of emulsified preparation of the dead animals' lungs into normal animals produced typical clinical symptoms of influenza attended by intensive general clinical phenomena, reduction of the survival period, and hemorrhagic condition in the lungs, especially in those of the rats.

To obtain a pure, virulent influenza virus with economy of laboratory animals, we proceeded in the following fashion: a 10-percent emulsion of the virus from the lungs of infected mice was prepared by the method described above. The lungs had been clogged on the fourth day after injection. The centrifugate of the emulsion was drawn off and filtered through a Zeitz filter after a preliminary passage of a mixture of 25 cubic centimeters of physiologic salt solution and 5 cubic centimeters of broth.

The entire filtered mixture, which contained the virus in a concentration of 1:150, was placed in a mechanical agitator for 2-3 hours until foam appeared. The concentration of the virus in the filtrate was then determined. We also studied the virulency of this suspension in various concentrations on susceptible animals. Moreover, the concentration of the virus in the foam and in an absorbent was also determined.

A sterile filter paper was lowered in a vertical position to the bottom of the vessel, to absorb the virus from the surface of the filtrate. After a short while, the section that has been saturated was cut off 0.5-1 cubic centimeters above the liquid level, and tested. Its contents were drawn off with a pipette or squeezed out with pincers.

The preparation obtained in this was was tested separately (the filtrate, the foam, and the paper) and in various concentrations after a secondary mechanical agitation and their sterility tested on Livinthal's and Field's media. The customary method of intranasal administration under ether was made on the mice with 2-3 minims (approximately 0.05-0.15 centimeter), and the rats with about one cubic centimeter. After infection, the animals were kept under observation, the mice for 6 days, and the rats for 15 days. Each animal that died during this period was dissected, revealing positive pathological degeneration (4+) in the lungs. The surviving animals were examined the same way. In all appearances the 36 house mice, 72 field mice, and 24 wild rats infected intranasally with the suspension of influenza virus under ether, both living and dead, all developed typical symptoms of the disease and a varying degree of pulmonary disease 2-4 days after the administration of the infective agent. In some cases, the animals became infected faster with larger dosage than with smaller dosage. Apparently, this was due to the concentration of the virus in the filtrate. An emulsion of lung tissues of these animals, when introduced into the respiratory system of normal animals, again caused a severe form of this disease and death of the animals.

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